

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
12 August 2004 (12.08.2004)

PCT

(10) International Publication Number
WO 2004/067732 A2

(51) International Patent Classification⁷: C12N 5/00,
C08F 220/00

(21) International Application Number:
PCT/CZ2004/000005

(22) International Filing Date: 26 January 2004 (26.01.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PV 2003-251 27 January 2003 (27.01.2003) CZ

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(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
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TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Euro-
pean (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR,
GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

Published:

— without international search report and to be republished
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: POLYMER CARRIERS WITH BONDED SACCHARIDES FOR IMMOBILIZATION OF BIOLOGICAL SYSTEMS

(57) Abstract: The solution concerns polymer carriers with bonded saccharides for immobilization of biological systems, where at the nonreducing end of a disaccharide is mannose or galactose that are covalently bonded to polymer matrix through various types of spacers, which enables a better contact of a saccharide molecule with receptors of biological systems.

WO 2004/067732 A2

Polymer carriers with bonded saccharides for immobilization of biological systems

5 Technical field

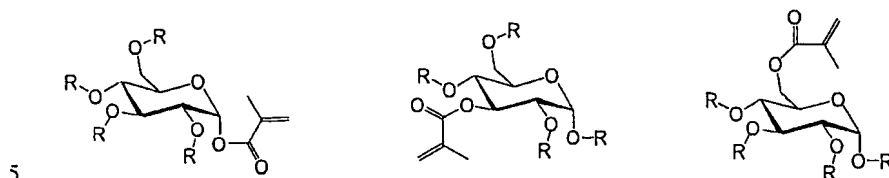
The invention deals with polymer carriers with covalently bonded saccharides for temporary immobilization of cells with receptors for mannose or galactose. The invention is also aimed at the preparation of these polymer carriers. Their main application is the use of keratinocyte receptors for cultivation of these cells and transfer of the cells onto burns. The invention is a
10 continuation of the patents of J. Labský et al. "Polymer carrier for keratinocyte cultivation" (Czech Patent Applications PV 1998-1803, PV 1999-1946, PV 1999-1947, WO 99645631 (PCT/CZ/99/00017)).

Background art

15 Biological systems such as some cell types, lectins and cell toxins, possess receptors, which enable temporary immobilization on polymer matrix surface with appropriate covalently bonded ligands. The temporarily formed receptor-ligand bond enables manipulation with the thus formed complex, such as separation, purification, cultivation or transport. Appropriate ligands for biological receptors are chemically bounded saccharides, such as mannose,
20 galactose or disaccharides (lactose). The almost identical hydroxy groups in molecules of mono- or disaccharides require special techniques for the preparation of polymerizable derivatives or activation of saccharide molecules for modification of suitable polymer carriers.

25 Most frequently are used polymerizable derivatives of mono- or disaccharides, where the hydroxy groups are protected, e.g., by acetylation, benzylation or benzoylation. Acetal derivatives of saccharides or analogous isopropylidene derivatives prepared by condensation with acetone can be converted by a suitable chemical modification to polymerizable derivatives. A variety of polymers with covalently bounded saccharides are described, where
30 polymerizable derivatives of saccharides are homopolymerized or copolymerized with hydrophilic (2-hydroxyethyl methacrylate, acrylamide, methacrylamide, 1-vinylpyrrolidin-2-one) or hydrophobic monomers (styrene, methyl methacrylate). Some types of saccharide monomers are described in the following brief survey. In literature are described saccharide derivatives, where the polymerizable group is bonded directly to the molecule of saccharide in

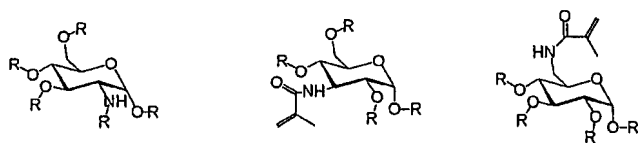
various positions (T. Kawasaki: US 4 465 827, 1984; D.K. Ray-Chaudhuri: US 3 356 652, 1967; Y. Iwakura et al.: J. Polym. Sci., Part A-1, 6, 1625-1632, 1968; T. Kawasaki: US 4 465 827, 1984)



Monomers are described where the polymerizable group is bonded to the saccharide molecule in positions 2 or 3 (S. Kimura et al: Makromol. Chem. 58, 232, 1962; S. Kimura et al: Makromol. Chem. 50, 155, 1961) or 6 (M. Imoto et al: Makromol. Chem. 53, 2120, 1962; N.P. Ivanova et al.: Zh. Prikl. Khim. 71, 114-118, 1998; W.A.P. Black et al.: Makromol. Chem. 117, 210-14, 1968; W.A.P. Black: Carbohydr. Res. 5, 362-5, 1967; J. Klein: Makromol. Chem. Rapid Commun. 6, 675, 1985; S. Kimura, M. Imoto: Makromol. Chem 50, 155 (1961), 53, 210, 1962).

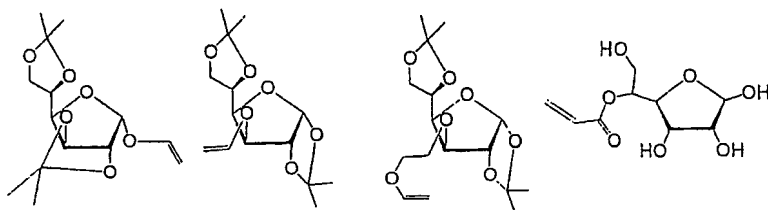
15 Amino derivatives of saccharides, where the amino group is bonded in various positions are also often used for the preparation of polymerizable derivatives (E. Fanton, C. Fayet: Carbohydr. Res., 240, 143-52, 1993, J. Klein: Makromol. Chem., Rapid Commun. 6, 675, 1985, B. Badley et al: Macromol. Chem. Phys. 198, 945-57, 1997, Suk Hyeong Cho et al: Korea Polym. J. 6, 188-192, 1998, R.L. Whistler et al: J. Org. Chem. 26, 1583, 1961).

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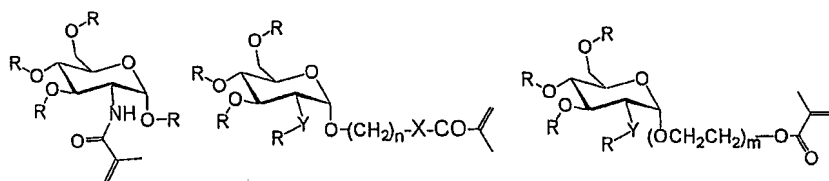


Both vinylic and methacryloylated derivatives of glycofuranoses can be found in literature (W.A.P. Black et al: J. Chem. Soc. 1963, 4433, W.A.P. Black: Makromol. Chem. 71, 189, 1964).

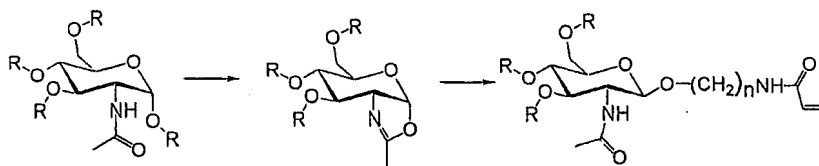
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Another, very frequently cited group of polymerizable monomers utilizes glycoside derivatives, often with a spacer between the saccharide molecule and polymerizable group. In the following illustrative examples, $n = 2-12$ and X and Y are $-O-$ or $-NH-$, $m = 2$ or 3 (K.S. Kim et al: Korea Polym. J. 4, 16-22, 1996, S.R. Sarfati et al: J. Chem. Soc., Perkin Trans. 1., 1990, 1065-70, B. Helferich, K.H. Jung, Hoppe-Seyler's Z. Physiol. Chem. 311, 54, 1958, R.L. Whistler et al: J. Org. Chem. 26, 1583, 1961, J. Klein, D. Herzog: Makromol. Chem. 188, 1217-32, 1987, H.P. Panzer, H.J. Roberts: J. Org. Chem. 26, 1583, 1961, Spaltenstein et al: J. Am. Chem. Soc. 113, 686-9, 1991, T. Nakaya et al: J. Macromol. Sci., Macromol. Rep. A 30, 349-55, 1993, J. Coulon et al.: Bioconjugate Chem. 9, 152-159, 1998).

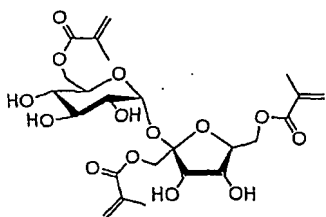


Oxazolines, which can be prepared from 2-acetamido-2-deoxysaccharides, are easily converted to 2-acetamido-2-deoxyglucosides, where a polymerizable group can be built-in in the aglycon. Here is given a specific preparation of ω -acrylamidoalkyl 2-acetamido-2-deoxy- β -D-glucopyranoside from 2-methyl-4,5-dihydro(3,4,6-tri-*O*-acetyl-1,2-dideoxy- α -D-glucopyranoso)[2,1-*d*][1,3]oxazole catalyzed by camphor-10-sulfonic acid ($n = 3, 6$) (S. R. Sarfati et al: J. Chem. Soc., Perkin Trans 1., 1990, 1065-70).



A variety of saccharide derivatives are described in literature, where the oxazoline skeleton of a saccharide can be transformed into a suitable precursor for the preparation of polymer glycosides (M. Okada, K. Aoi: Jpn. Kokai Tokkyo Koho JP 08259587, 1996), Jun-Ichi Kadokawa et al: Macromolecules 30, 8212-17, 1997).

A saccharose derivative is given as an example of disaccharides with one or more polymerizable groups (T. Furuike et al: Macromolecules 1995, 28, 7241-7, J. Chen et al: WO 98/51694), N. D. Sachinvala et al: Carbohydr. Res. 218, 1991, 237-45).



5

An aminosaccharide can be attached to a soluble polymer by the reaction with an active ester, e.g. according to J. F. Bridges, R. Duncan, J. Kopeček: Int. J. Pharm. 44, 213-223, 1988.

10 The brief enumeration of polymerizable monomers, which is by no means exhausting, already shows a great variability in the preparation of polymer carriers, where the saccharide units built-in in a homopolymer or copolymer with other monomers, should fulfil the function of a ligand for receptors of biological systems. Some drawbacks of the thus prepared polymers should be here mentioned.

15

Protected hydroxy groups often resist quantitative deblocking. Varying the saccharide content in the copolymer considerably changes swelling characteristics of the polymer and hence also mechanical properties of the polymer.

20 A distinct disadvantage of polymer carriers utilized as cultivation supports for immobilization or separation of biological substances and formed as copolymers of saccharide monomers with protected hydroxy groups and hydrophilic or hydrophobic comonomers is the fact that the protecting groups must be removed. The polymers or copolymers prepared using the saccharides where the protecting groups were removed prior to the polymerization show also
25 problems - poor solubility of saccharide monomers in organic solvents and comonomers, the necessity to polymerize mostly in water, poor mechanical properties of the formed copolymers.

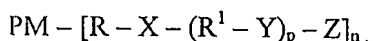
A variable content of saccharide units in the polymer significantly affects mechanical
30 properties of the polymers to such extent that they cannot be used as cultivation supports.

Another disadvantage is that the removal of protecting groups is often not quantitative and the residual groups may affect the polymer structure.

The present invention suggests the use of hydrophilic materials as matrices, both synthetic and natural, where specific ligands are bonded on modification of the carrier surface or activation of suitable saccharide derivatives and subsequent reaction with the matrix. Under these conditions, the reaction proceeds in surface layers of the matrix and mechanical properties of the polymer formed are almost identical with those of the starting material and the amount of surface-bonded saccharides can be controlled.

Disclosure of the invention

The subject of the present invention is the polymer carriers with bonded saccharides mannose, galactose or disaccharides, where at the nonreducing end of the disaccharide is mannose or galactose, which are covalently bonded to polymer matrix using various types of spacers, whereby polymer matrices are made suitable for cultivation of keratinocytes or for temporary immobilization of biological systems with receptors for mannose and galactose of the general formula



where PM is a hydrophilic crosslinked polymer prepared by radical polymerization of a polymerization mixture containing: (a) 1-99 wt.% of a monomer or monomer mixture, (b) 0.1-10 wt.% of a crosslinker or crosslinker mixture, (c) 0.01-10 wt.% of a radical initiator and/or (d) 0.1-40 wt.% of a solvent or solvent mixture

and the general structure

$-[R - X - (R^1 - Y)_p - Z]_n$ formed by subsequent modification of polymer matrix, where

R is independently selected from the group: covalent bond, $-(CH_2)_a-$, $-OCH_2CH_2-$, $-(OCH_2CH_2)_b-$, $-C_6H_4-O-$, $-C_6H_4-CO-$, $-NH-C_6H_4-CO-$, $-C_6H_4-NH-$, $-O-C_6H_4-O-CH_2CH_2O-$, X is independently selected from the group: covalent bond, $-O-$, $-NR^2-$, $-CO-O-$, $-CO-NH-$, $-NH-CO-$, $-O-CO-O-$, $-NH-CS-NH-$, $-NH-CO-NH-$, $-O-NH-CH_2CH_2CONH-$, where R^2 is $-H$, alkyl C_1 -, C_4 or acetyl,

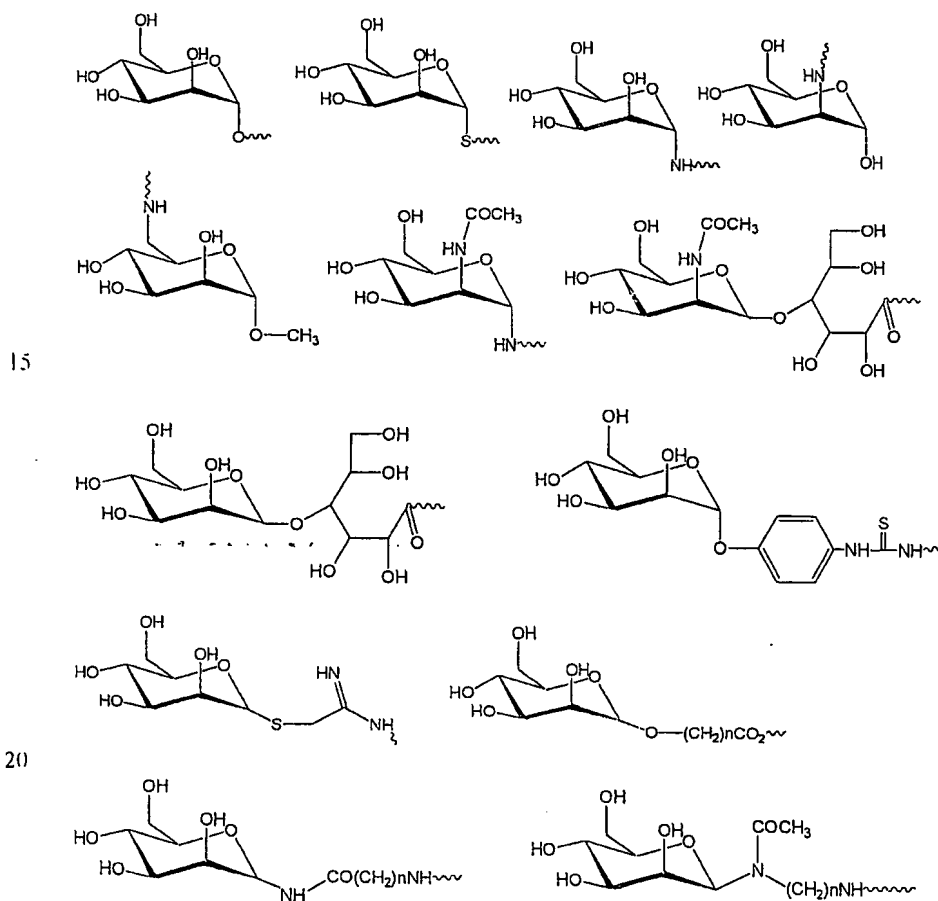
R^1 is independently selected from the group: covalent bond, $-(CH_2)_a-$, $-OCH_2CH_2-$, $-C_6H_4-O-$, $-(OCH_2CH_2)_b-O-$, $-C_6H_4-CO-$, $-NH-C_6H_4-CO-$,

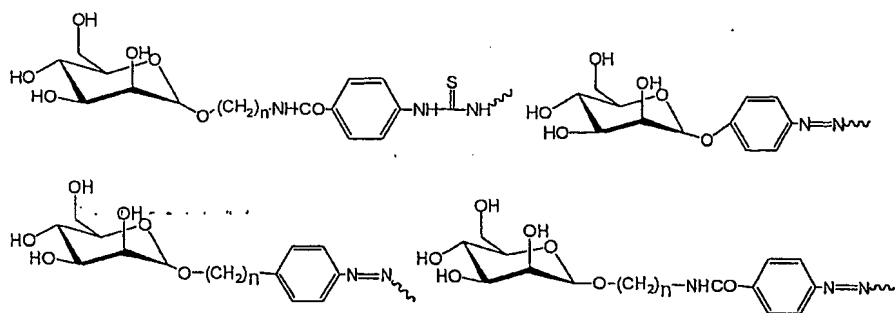
Y is independently selected from the group: covalent bond, $-N(COCH_3)-$, $-NH-CS-NH-C_6H_4-O-$, $-NH-CS-NH-(CH_2)_a-C_6H_4-O-$, $-NH-CS-NH-(CH_2)_a-O-C_6H_4-O-$,

5 $-NH-CS-NH-(CH_2)_a-C_6H_4-O-$, $-NH-C_6H_4-O-$, $-NH-C_6H_4-CO-$

where the subscript a ranges from 1 to 12, b from 1 to 200, p is 0-20, and subscript n is selected to ensure the saccharide concentration in the range from 1×10^{-4} to 0.3 g per gram of polymer matrix,

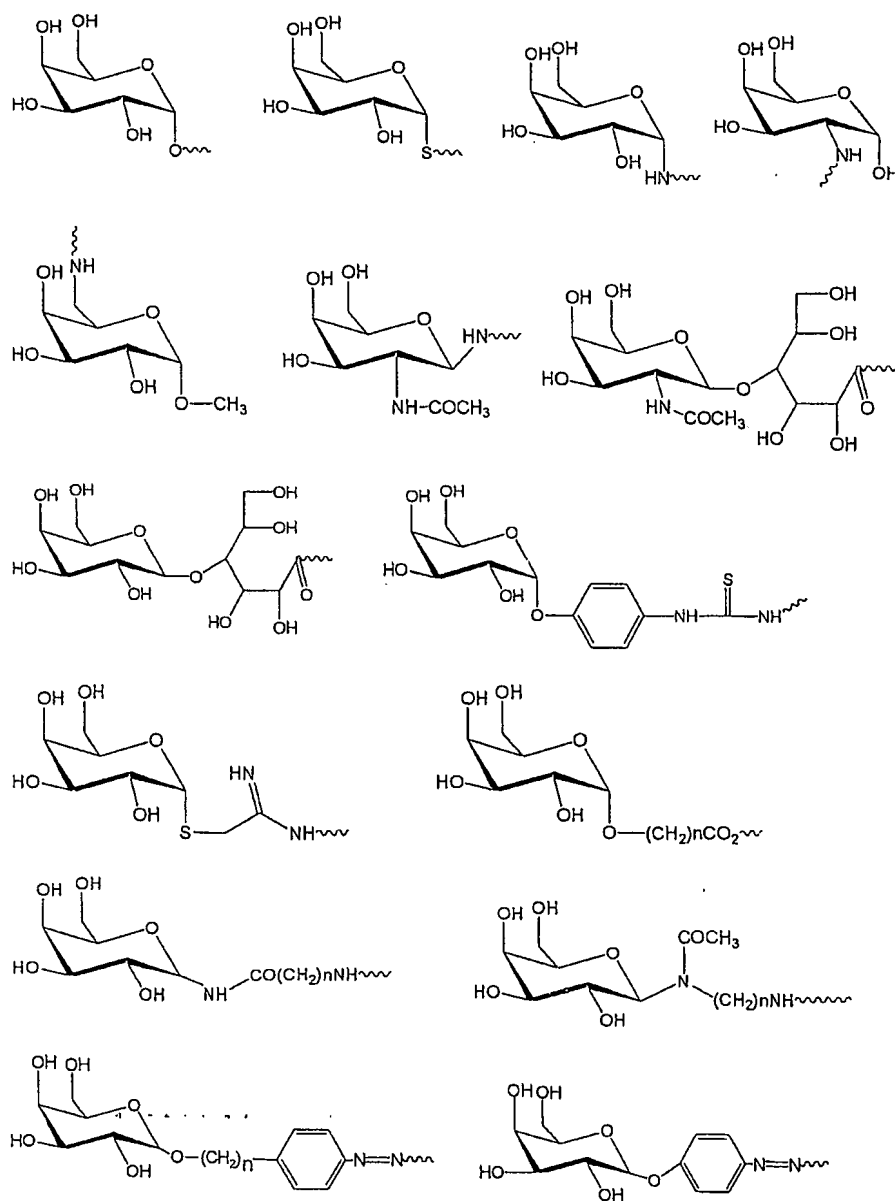
10 Z is independently selected from the group of mannose, galactose and lactose derivatives, whose structure formulae are given; the wavy line shows the attachment of the saccharide molecule. The mannose derivatives are as follows





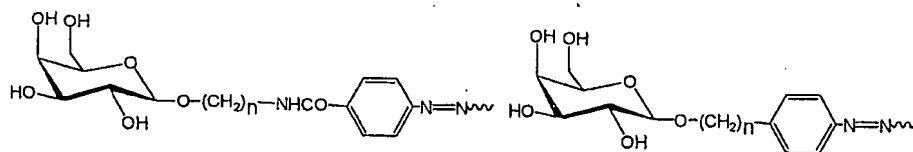
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The galactose derivatives are as follows

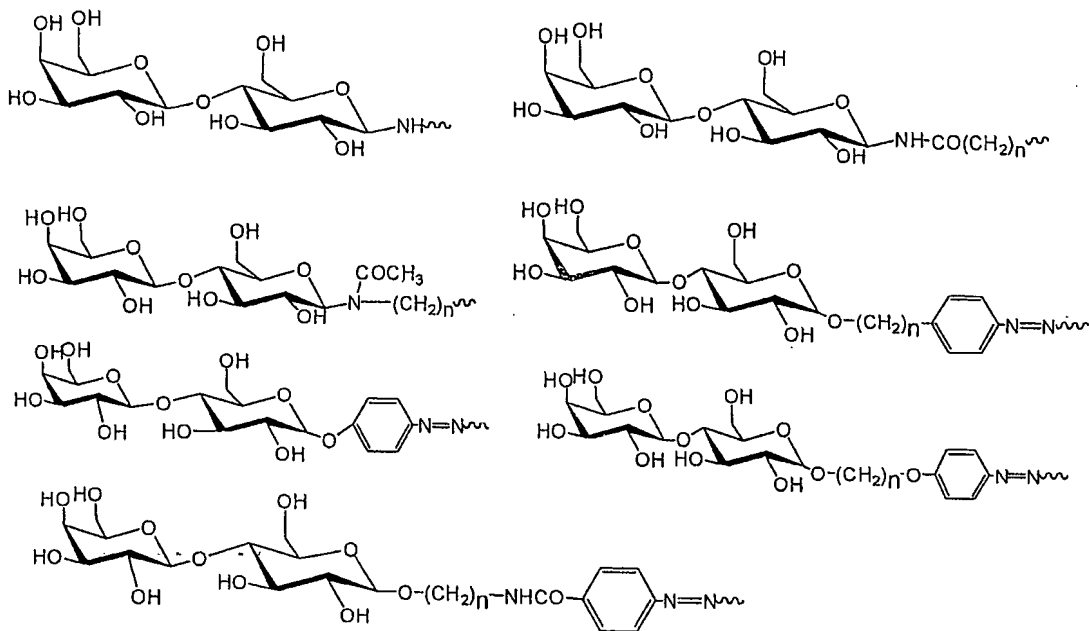


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The lactose derivatives are as follows



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The basic materials for polymer carriers according to the invention are hydrophilic polymer matrices, which are surface-activated and, subsequently, saccharides are activated.

Polymer matrix

- 10 To advantage, activation of surface layers of polymer carriers is associated with application of spacers because in interactions of polymer systems with biological materials the antenna effect mostly operates, which enables the receptor contact with a polymer ligand.

Polymer carriers suitable for preparation of cultivation supports by their surface modification may be of natural (cellulose, alginates) or synthetic origin (poly(vinyl alcohol), poly(2-hydroxyethyl methacrylate) etc.). The suitability of a polymer is determined by several parameters such as swelling, mechanical properties and, last but not least, by the presence of reactive groups such as hydroxy, amino or carboxy groups. Biocompatibility, chemical inertness, stability in biological media, insolubility, sterilizability and zero extractability of polymer matrices are other necessary requirements for their applications.

20

Suitable monomers for preparation of polymer carriers according to the invention are 2-hydroxyethyl acrylate, 2-hydroxyethyl methacrylate (HEMA), 2-(2-hydroxyethoxy)ethyl acrylate, 2-(2-hydroxyethoxy)ethyl methacrylate (DEGMA), tri-, tetra- and poly(ethylene glycol) monoacrylates and methacrylates, glycerol acrylate and methacrylate, 2-hydroxypropyl acrylate and methacrylate, ω -hydroxyalkyl acrylates and methacrylates, *N*-(ω -hydroxyalkyl)acrylamides and -methacrylamides, *N*-(ω -aminoalkyl)acrylamides and -methacrylamides, glycidyl acrylate and methacrylate, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]acrylamide and -methacrylamide, acrylic and methacrylic acid, ω -acrylamido- and ω -methacrylamidoalkanoic acids, acryloylated and methacryloylated α -amino acids, 4-vinylbenzoic acid, acrylamido- or methacrylamidobenzoic acids, vinyl acetate, *N*-alkylacrylamides and -methacrylamides. The enumeration of the monomers is naturally incomplete and by no means limits the use of other monomers usual in the field.

The following crosslinkers can be used for the preparation of polymer matrices according to the invention: ethylene diacrylate and dimethacrylate, diethylene glycol and oligo(ethylene glycol) diacrylates and dimethacrylates, *N,N*-ethylenediacrylamide and *N,N*-ethylenedimethacrylamide, 1,3-divinylurea, 1,1'-divinyl-3,3'-(ethane-1,1-diyl)di(pyrrolidin-2-one), 2,3-dihydroxybutan-1,4-diyl diacrylate or dimethacrylate and also other crosslinkers usually used in the field.

20

For initiation of polymerizations, thermal radical initiators are suitable such as azo compounds, diacyl peroxides and other types of peroxo compounds, photoinitiators generating radical by the action of UV radiation or redox initiators, which generate radicals by an oxidation-reduction reaction.

25

In some cases it is convenient to use a solvent in the preparation of polymer matrix, such as water, alcohols (methanol, ethanol, glycol, glycerol), dimethylformamide, dimethyl sulfoxide, poly(ethylene glycol)s, esters of aliphatic acids, ethylene glycol monomethyl or dimethyl ether and also other common solvents used in the field.

30

The forms and shapes of polymer matrices may be different. Common cultivation supports are prepared in moulds for film casting, where polymerisations can be performed also in the presence of solvents. For some purposes it is more convenient to prepare spongy polymers,

foams, microparticles or beads. The techniques are well known and commonly used in the field and, therefore, are not further discussed here.

Activation of polymer matrix surface

- 5 By radical polymerization, polymer matrix is formed with the idealized structure $PM-(R^a)_p$, where PM represents the structure of the crosslinked carbon chain formed by radical polymerization and the R^a group is any functional group, which is a constituent of the used monomers or comonomers. The structure of this part of the matrix corresponds to the polymerization mixture composition and the functional groups correspond to the structure of
- 10 the starting monomer.

R^a is most frequently -OH, -COOH, -CH₂CH₂OH, -CH₂CH(OH)CH₃, -NH₂, -CH₂NH₂, -C₆H₄COOH, -C₆H₄OH, -C₆H₄OCH₂CH₂OH, -CONH(CH₂)_nCOOH, -C₆H₄NH₂, -COO(CH₂)_nCOOH, -CONH(CH₂)_nNH₂. This list of reactive groups is only illustrative; a variety of other possible groups are available, which must be included in the list.

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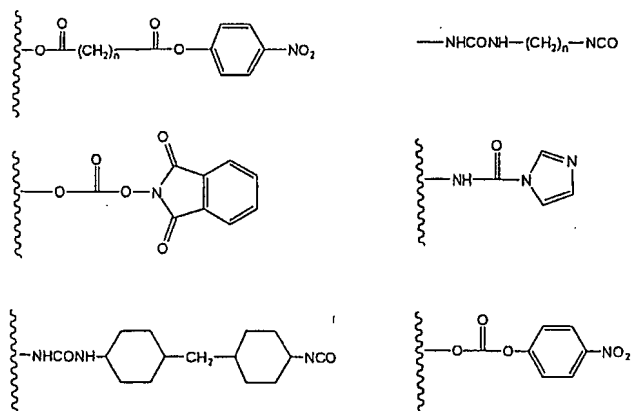
- All the functional groups denoted R^a are not utilized for modification reactions leading to binding of spacers and subsequent reaction with activated saccharides. The reactions occur in matrix surface layers and do not significantly affect mechanical properties of the matrix. In utilizing accessible reactive groups of the matrix and subsequent reaction with saccharides, a
- 20 surface phenomenon appears called the orange effect. The effect consists in that on attachment of substances of the nature pronouncedly different from the matrix structure (saccharide), the matrix surface bursts during swelling due to different swelling characteristics of the ligand and matrix.

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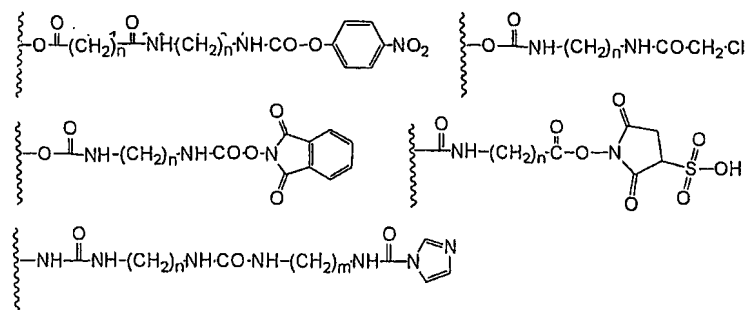
- The modified matrix has the idealized structure $PM-(R^b-X^b)_x$, where the general formula $-R^b-X^b$ represents the matrix surface structure after modification reaction and, clearly, its concentration is lower than the original introduced groups generally denoted $-R^a$. The symbol X^b represents reactive groups or a spacer as described below. Possible repetition of the
- 30 modification reaction using the same or different compounds (e.g., diazotation and azo coupling, formation of an active ester) is included in the general representation.

Activation of amino and hydroxy groups

- Activation of a hydroxy or amino group can be accomplished by the reaction with bifunctional reactive systems, e.g. dichlorides or chloride-esters of dicarboxylic acids, such as dicarboxylic acids of the general formula HOOC-Q-COOH , where Q represents a bifunctional aliphatic chain, branched aliphatic chain, cycloalkanediyl, cycloalkenediyl, phenylene, furandiyl, oxydiethylene, diisocyanates of the general formula OCN-T-NCO and diisothiocyanates SCN-T-NCS , where T represents a divalent aliphatic chain, cyclohexane-1,4-diyl, methylenedi-1,4-phenylene, oxydi-1,4-phenylene, methylenedicyclohexane-1,4-diyl. Another possibility of activation of hydroxy or amino groups is the use of cyanogen bromide, phosgene, diphosgene, chlorocarbonic esters of aliphatic alcohols, branched aliphatic alcohols, cyclic alcohols, 1,1'-carbonyldiimidazole or other derivatives of carbonic acid commonly used in the field. The thus modified matrix surface selectively reacts in another step with an aliphatic amino group (J. Drobník, J. Labský, H. Kudvasrová, V. Saudek, F. Švec: Biotechnol. Bioeng. 2, 487, 1982, J. Drobník, J. Kálal, J. Labský, V. Saudek, F. Švec: Czech Pat. 204 190, US 4 245 064, 1979, K. Takeda, H. Ogura: Synth. Commun. 12, 213, 1982, K. Takeda et al.: Tetrahedron Lett. 24, 4569, 1983, N. Nimura et al.: Anal. Chem. 58, 2372, 1986).

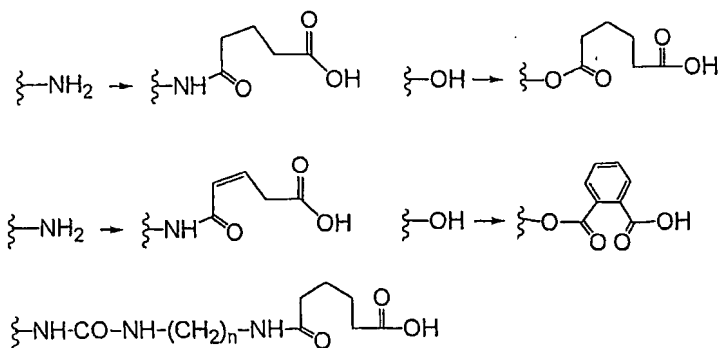


- 20) Surface activation can be repeated, hence the resulting structures can be, e.g.,

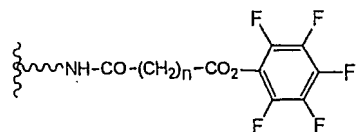


Formation and activation of carboxylic group

Carboxylic groups can be built-in into polymer carrier using an appropriate polymerizable monomer, such as acrylic, methacrylic acid, maleic anhydride, ω -acrylamidoalkanoic acids, ω -methacrylamidoalkanoic acids or by additional modification of other reactive groups. Modification of polymer surface by the reaction of cyclic anhydrides with amino or hydroxy group of the carrier affords carboxylic groups



- Another possibility is the reaction of polymer matrix with an appropriate derivative of dicarboxylic acid such as dichloride or active ester. Suitable alcohols for the active esters are, e.g., 4-nitrophenol, pentachlorophenol, pentafluorophenol, 2,4-dinitrophenol, *N*-hydroxysuccinimide, *N*-hydroxyphthalimide, 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonic acid and other compounds used in the field. When using excess reagent, predominantly one group of dicarboxylic acid reacts; the other can be utilized for the reaction with an aliphatic amino group, e.g. of a saccharide.

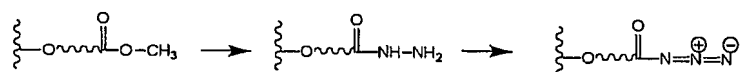


- Activation of the carboxylic group is possible in several ways. Its transformation into acid chloride by the action of, e.g., thionyl chloride, phosphorus trichloride, phosphorus pentachloride, oxalyl chloride is easy. A necessary requirement is that the polymer carrier be inert (A. K. Ghosh et al.: Tetrahedron Lett. 33, 2781, 1992, J. P. Greenstein, M. Winitz: Chemistry of the Aminoacids. J. Wiley, New York 1961)



Another possible activation of the carboxyl is the transformation of an acid ester to acid hydrazide and further to azide. The reaction is performed by the action of hydrazine, most frequently on an acid ester and by subsequent reaction with sodium nitrite or a nitroso compound (J. P. Greenstein, M. Winitz: Chemistry of the Aminoacids. J. Wiley, N.Y, 1961). The hydrazide can be also prepared by the carbodiimide-activated reaction of an acid with hydrazine (J. Drobňík, J. Kálal, J. Labský, V. Saudek, F. Švec: Czech Pat. 204 190, US 4 245 064, 1979).

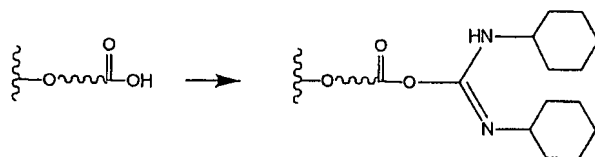
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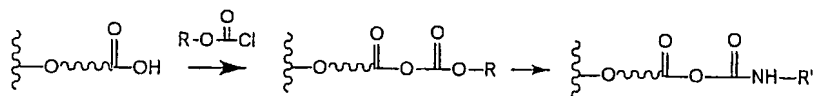
A frequently used activation of the carboxyl group, probably the mildest for polymer carriers, is performed by activation with carbodiimides or analogous compounds, where active ester is an intermediate. The technique is perfectly elaborated, offering the reaction proceeding in both aqueous and organic media; the yields of the condensation reaction often range above 80 %. As an example, the intermediate is given formed by the reaction of the carboxyl with dicyclohexylcarbodiimide (M. K. Dhaon et al: J. Org. Chem. 47, 1962, 1982, J. C. Sheehan et al: J. Am. Chem. Soc. 95, 875, 1973).

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An elegant procedure affording good yields is the mixed anhydride method, which consists in the reaction of alkyl chlorocarbonates with the carboxyl group in the presence of a tertiary amine (J. P. Greenstein, M. Winitz: Chemistry of the Aminoacids. J. Wiley, N.Y, 1961). The acylation proceeds selectively only with the used acid, carbonic acid derivatives do not take part in the reaction.



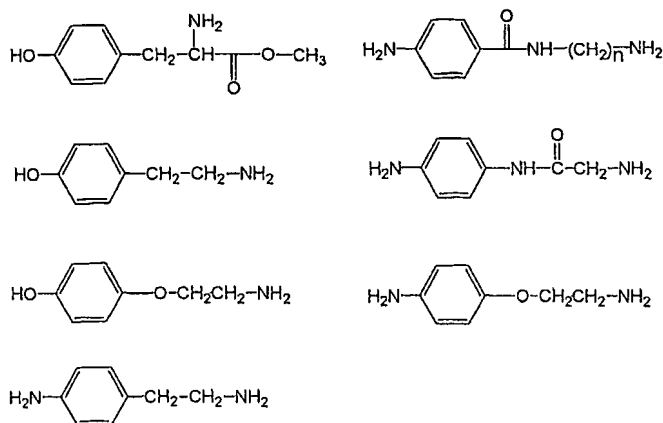
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An advantage of the reaction is that the by-products are alcohol (for R = ethyl, it is ethanol) and carbon dioxide.

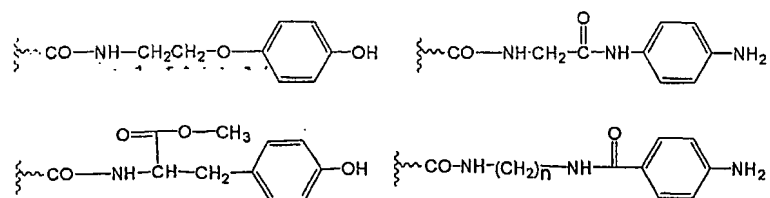
Modern reagents for carboxyl activation, e.g. water-soluble *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide (EDC) (R. R. Fischer: J. Biol. Chem. 256, 8963, 1981, Abdelmoty et al: Lett. Pept. Sci., 1, 57, 1994, R. Knorr et al.: Tetrahedron Lett. 30, 1927, 1989, V. Dourtoglou et al.: Synthesis 572, 1984) or condensation agents based on *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetra- or hexafluorophosphate (HBTU), *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU), (benzotriazol-1-yloxy)dipiperidinomethylum hexafluorophosphate (HBPIP) et al. are very effective, but the methods are not widespread due to high prices of the reagents. Worth mentioning is also *N*-ethyl-5-phenylisoxazolium-3'-sulfonate (Woodward reagent), which easily activates carboxyl and is used for amide bond formation (R. B. Woodward et al: J. Am. Chem. Soc. 83, 1007-9, 1961, R. B. Woodward et al: J. Am. Chem. Soc. 83, 1010-12, 1961) or 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) (B. Belleau et al: J. Am. Chem. Soc. 90, 1651, 1968, J. Klein, D. Herzog: Makromol. Chem. 188, 1217-32, 1987, R. Roy: Top. Curr. Chem. 187, 242-271, 1997), which reacts in an analogous way. The list of the reagents is not exhaustive; also analogous reagents well known in the field can be used.

20 Activation by aromatic amines and phenols

A variety of reactions can be used for activation of polymer matrix with aromatic amines or phenols, which make it possible to build-in an aromatic system suitable for diazotation and azo coupling. Examples of the compounds used are as follows.



The resulting polymer matrices suitable for azo coupling with diazonium salts have, e.g., the following structure



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Activation of saccharides

D-Mannose and D-galactose or their 2-amino-2-deoxy derivatives often occur in natural materials. These saccharide units are frequently located at the nonreducing ends of saccharide chains and are key saccharides for interactions with active centres of biological systems. Saccharides bonded to polymer matrices have the α or β structure; most often they are mixtures of the stereoisomers in dependence on the used reactions. The structure details, however, cannot be determined in the matrix and are not a hindrance in the use of matrices.

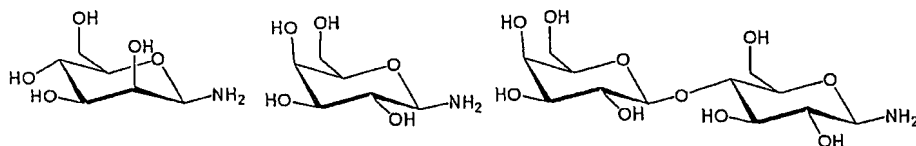
The described modifications of saccharides frequently make use of protecting groups for hydroxy groups of saccharides. For the sake of brevity, they are omitted in structural formulae. Clearly, the final modification of the matrix surface implies the removal of the protecting groups. The methods for the purpose are standard and are not given here.

Under the reducing part of disaccharides is understood the part of disaccharide containing the hemiacetal grouping in the pyran ring of the saccharide, which easily undergoes hydrolysis and the resulting aldehyde group is subject to consecutive reactions, such as reductive amination.

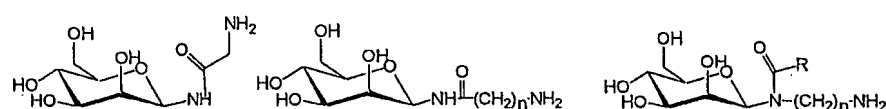
Amino derivatives of mannose and galactose

In addition to commercially available 2-amino-2-deoxy-D-mannose and -galactose, mannosyl-, lactosyl- and galactosylamine can be used, which are easily prepared from mannose, lactose or galactose by the reaction with ammonia, ammonium hydrogencarbonate or ammonium carbonate (L. M Likhoshesterov et al: Izv. Akad. Nauk, Ser. Khim. 8, 1461, 2000, R. Duncan et al: J. Controlled Release 10, 51-63, 1989, T. Rademacher, I.D. Manger: US 5,280,113).

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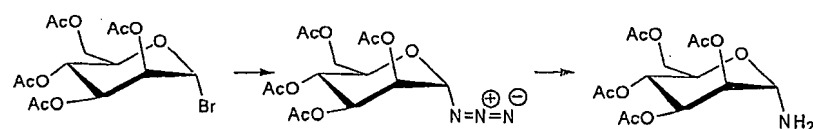
These amino derivatives are rather unstable but the formation of the amide bond stabilizes them. The derivatives appropriate for modification of polymer carriers are, e.g., ω -amino-*N*-(D-mannopyranosyl)alkanamide or *N*-(ω -aminoalkyl)-*N*-(D-mannopyranosyl)alkanamide (J. J. Garcia-Lopez et al: Chem. Eur. J., 5, 1775-1784, 1999, A.Ya. Chernyak et al.: Carbohydr. Res. 223, 303-9, 1992) or analogous derivatives of galactose:



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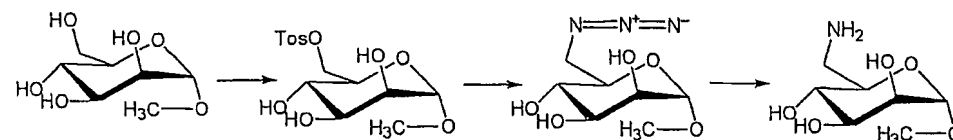
Mannosylamine, galactosylamine and lactosylamine can also be prepared by reduction of the corresponding azide. In this way, also their O-acetylated or O-benzoylated derivatives are prepared, which, after reduction and attachment to polymer matrix, can be easily deacetylated with a methanolic solution of sodium methanolate.

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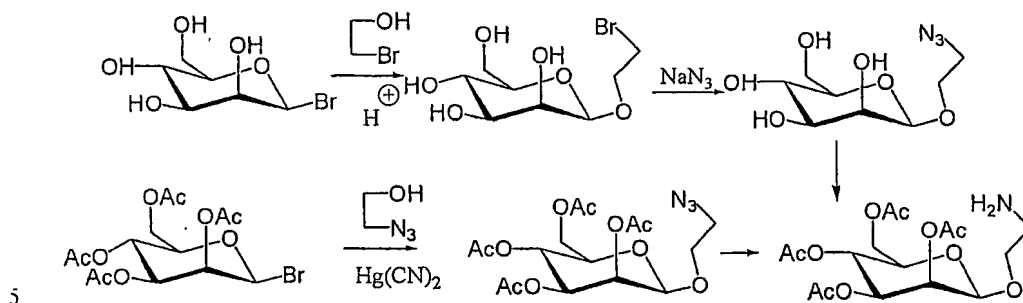


Methyl 6-amino-6-deoxy-D-mannopyranoside, accessible from methyl α -D-mannopyranoside by tosylation and subsequent reaction with sodium azide and reduction, was also used for the mannosylation reaction (A. L. Cimecioglu et al: Macromolecules, 30, 55-6, 1997, R. Roy et al: Glycoconjugate J. 15, 251-63, 1998).

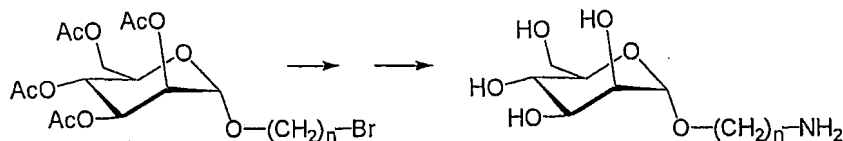
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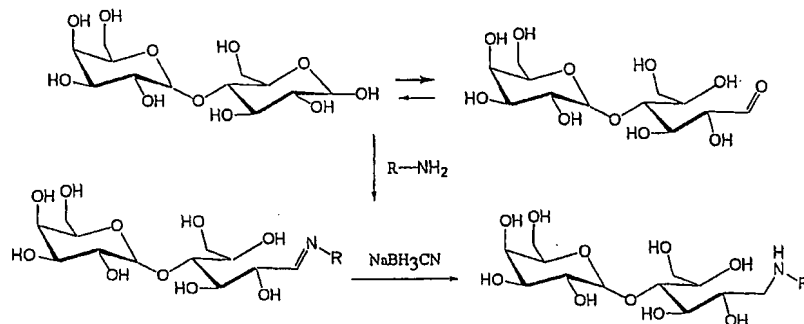
A frequently used mannose derivative is 2-aminoethyl β -D-mannopyranoside, which can be prepared in several ways, e.g. (A.Ya. Chernyak et al: Carbohydr. Res. 223, 303-9, 1992, A.Ya. Chernyak et al: Glycoconjugate J. 8, 82-9, 1991, Patel et al: J. Org. Chem. 2002, 79-86, J. M. Kim, R. Roy: J. Carbohydr. Res. 16, 1281-92, 1997):



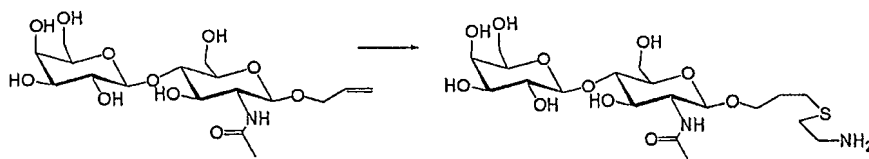
The reaction can be extended to halogenated alcohols of the general formula



- 10 The reductive amination of the aldehyde group in a reducing disaccharide can also be used for the preparation of saccharide amines.



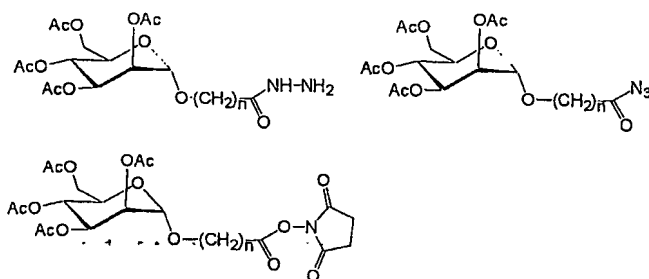
- 15 Another possibility, often used in the field, is the addition of 2-aminoethane-1-thiol onto the double bonds of acrylamide or allyl derivatives, e.g. (M.G. Baek et al: Biomacromolecules 1, 768-770, 2000):



Carboxy derivatives of saccharides

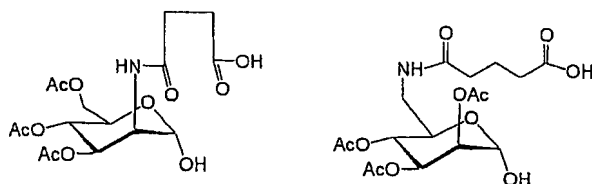
A variety of glycosides were prepared, where a carboxyl group is present in the aglykon, e.g. methyl 8-[1-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)]octanoate (B. M. Pinto et al: Carbohydr. Res. 124, 313-18, 1983, K. Wada et al: J. Carbohydr. Chem. 13, 941-65, 1994).

5 The ester can be transformed into derivatives appropriate for the reaction with activated matrix (carboxylic acid, active ester, hydrazide, azide) (R. U. Lemieux, D. A. Baker: Can. J. Biochem. 55, 507-12, 1977).



14)

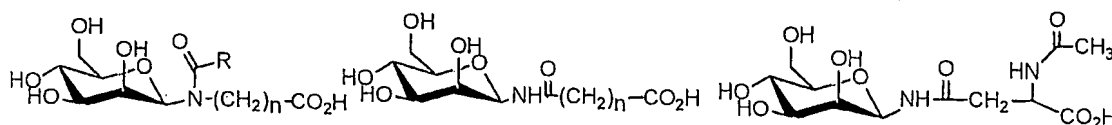
The carboxyl group can be generated at another site of saccharide molecule, e.g. by the reaction of an amino group with cyclic anhydrides of dicarboxylic acids:



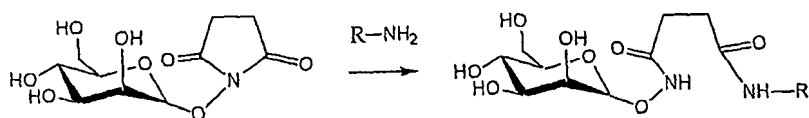
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Mannosylamine and galactosylamine can be easily transformed into derivatives that can be used for the reaction with activated matrix, such as (T. Rademacher: US 5 280 113, I.D. Manger: EP 0413675, H. Parot Lopez et al: Tetrahedron Lett. 33, 209-13, 1992, D. Vetter: Bioconjugate Chem. 6, 319-22, 1995, J. R. Rasmussen: US 5 283 353):

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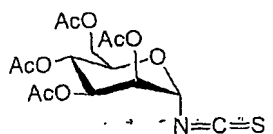
25 *N*-succinimidyl glycopyranosides are saccharide derivatives, which, in the reaction with the matrix amino group, form a straight-chain spacer (J. M. Kim, R. Roy: J. Carbohydr. Res. 16, 1281-92, 1997, S. Cao, F. D. Topper, R. Roy: Tetrahedron 51, 6679-86, 1985):



5 Isothiocyanates of saccharides

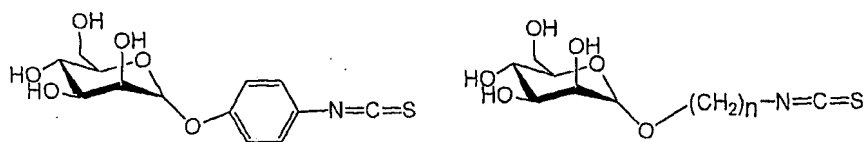
Saccharides modified with isothiocyanate groups are widespread in the synthesis of glycosylated derivatives. Peracetylated mannosyl isocyanate forms a crystalline derivative (B. König et al: Tetrahedron Lett. 39, 2307-10, 1998, J. Kerekgyarto et al: Carbohydr. Res. 297, 1997, 107-15)

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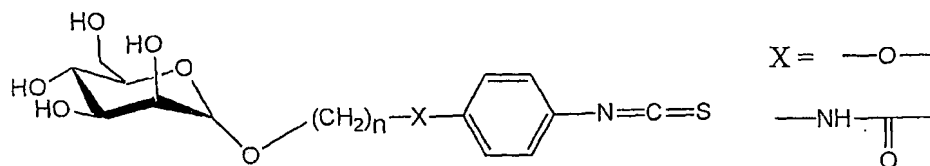
4-Isothiocyanatophenyl α -D-mannopyranoside can be prepared from 4-nitrophenyl α -D-mannopyranoside by reduction of nitro group and consecutive reaction with thiophosgene. An analogous derivative was prepared from ω -aminoalkyl α -D-mannopyranoside (C. R. Broom et al: Methods Enzymol. 28, 212-19, 1972, D.F. Smith et al: Methods Enzymol. 50, 163-75, 1978, S. Kotteer: J. Chem. Soc., Perkin Trans. 1, 2193, 1998)

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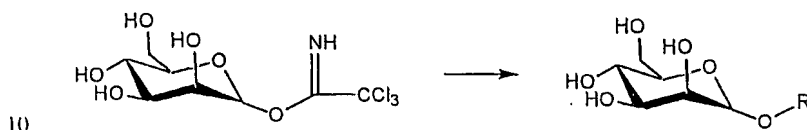
Isothiocyanates were derived that contain a spacer between the aromatic ring and saccharide, e.g. (M. Andersson et al: Bioconjugate Chem. 4, 246-9, 1993):

25



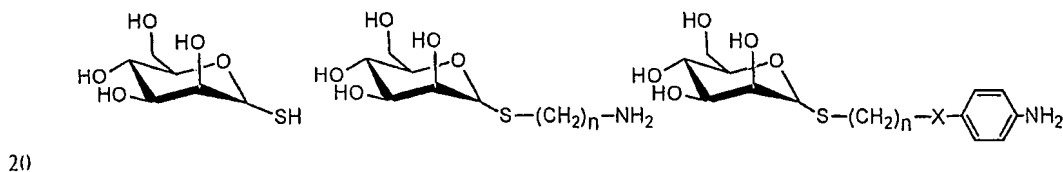
Trichloroacetimidates of saccharides

In the saccharide chemistry, great attention is rightly paid to trichloroacetimidate chemistry, where high yields and simple preparation guarantee wide spreading of the method for preparation of glycosidic derivatives (R. R. Schmid: *Prep. Carbohydr. Chem.*, pp. 283-312, S. Hanessian, Ed., S. Dekker: New York 1997). The reaction with a hydroxy group of the matrix is schematically outlined:

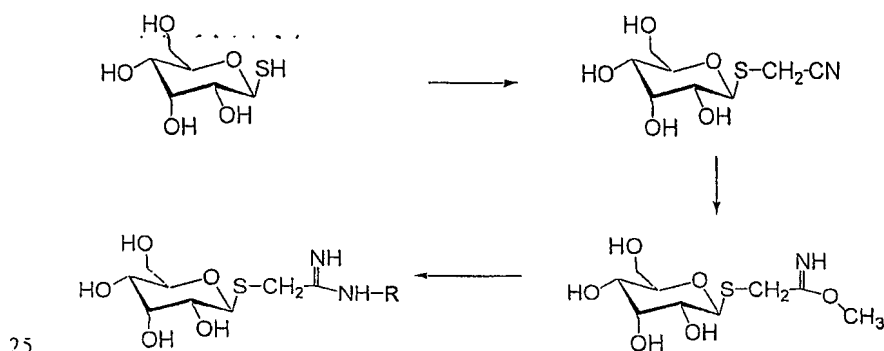


Thio derivatives

A relatively easy preparation and a considerably higher reactivity of the SH group make it possible to extend the assortment of reactive saccharide derivatives (P. H. Weigel et al: *J. Biol. Chem.* 254, 10830-8, 1979, S. Chipowsky et al: *J. Carbohydr. Res.* 31, 339-346, 1973, G. Pastuch et al: *Tetrahedron Lett.* 41, 9923, 2000, F. M. Ibatullin et al: *Synthesis* 419, 2001).

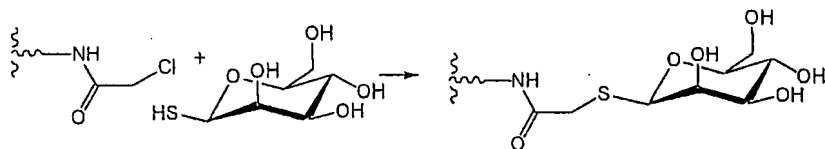


It is necessary to mention also amidines, which can be easily prepared by the reaction of 2-imino-2-methoxyethyl 1-thioglycopyranosides with amines (Y. C. Lee, C.P. Stowell, M. J. Krantz: *Biochemistry* 15, 3956, 1976)



Thio derivatives react in alkaline medium easily with haloacetyl derivatives, such as (H. W. I. Peerlings et al: Eur. J. Org. Chem., 1879-86, 1998)

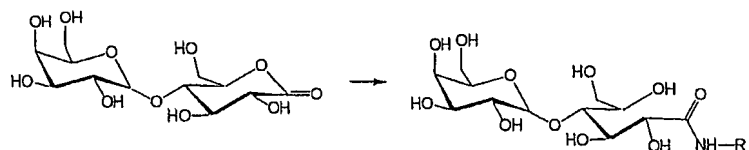
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Lactones

β -Galactopyranosyl-(1 \rightarrow 4)-D-glucono-1,5-lactone reacts with an aliphatic amino group under the formation of amide (K. Aoi et al: Macromolecules 28, 5391, 1995). Di- and oligosaccharides that contain mannose in the nonreducing part react analogously.

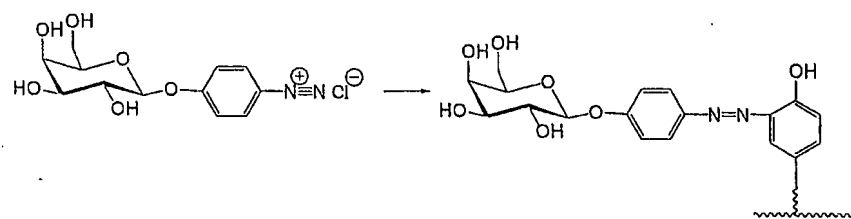
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15 Benzenediazonium derivatives

Aminophenyl derivatives of saccharides and disaccharides, easily convertible to diazonium salts, can be azo-coupled with biological materials, which contains groups (tyrosine) undergoing azo coupling. This fact can be utilized for binding appropriate derivatives to matrices with attached phenols, e.g. (C. P. Stowell, Y. C. Lee: Adv. Carbohydr. Chem. 37, 225-281, 1980, M. Wickek et al: PCT Int. Appl. WO 2000027814 A1, 2000):

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The brief enumeration of possible methods of preparation of polymer matrices with attached mono- and disaccharides is only illustrative and by no means limits the use of analogous

25

reactions for the preparation of the matrices that can be used for temporary immobilization of cells with mannose and galactose receptors.

Examples

5 Example 1

95 g (0.5 mol) of tetraethylene glycol is mixed with sodium methanolate (from 11.5 g sodium and 200 ml methanol) under cooling and methanol is evaporated in vacuum. To the reaction mixture diluted with 300 ml of acetone is dropped 50 g (0.5 mol) of methacryloyl chloride during 30 min at 0 °C under cooling. The mixture is left standing for 24 h, the precipitated
10 sodium chloride is filtered off and volatiles are evaporated in vacuum. The residue contains 59 % tetraethylene glycol, 34 % tetraethylene glycol monomethacrylate and 5.9 % tetraethylene glycol dimethacrylate.

Example 2

15 75 g (0.5 mol) of triethylene glycol is mixed with sodium methanolate (from 11.5 g sodium and 200 ml methanol) under cooling and methanol is evaporated in vacuum. To the reaction mixture diluted with 300 ml of acetone is dropped 50 g (0.5 mol) of methacryloyl chloride during 30 min at 0 °C under cooling. The mixture is left standing for 24 h, the precipitated sodium chloride is filtered off and volatiles are evaporated in vacuum. The residue contains
20 57 % triethylene glycol, 26 % of its monomethacryloyl derivative and 5.1 % of the dimethacrylate.

Example 3

A mixture of 40 ml of 2-hydroxyethyl methacrylate, and 25 ml of the product mixture containing tetraethylene glycol monomethacrylate (Example 1) and 1 ml of Darocur 1173
25 (Ciba) was bubbled with argon for 10 min and poured into a mould for preparation of films of 1.6 mm thickness. The polymerization mixture was irradiated with Philips UV lamps of total output 160 W from a distance of 20 cm for 20 min. The obtained film was extracted with 30% ethanol in distilled water and subsequently with distilled water and then dried.

30

Example 4

A mixture of 30 ml of 2-hydroxyethyl methacrylate, 0.3 ml ethylene dimethacrylate and 0.6 ml of Darocur 1173 (Ciba) was bubbled with argon for 10 min and poured into a mould for preparation of films of 1.6 mm thickness. The polymerization mixture was irradiated with

Philips UV lamps of total output 120 W from a distance of 20 cm for 20 min. The obtained film was extracted with 30% ethanol and water.

Example 5

- 5 A mixture of 40 ml of 2-hydroxyethyl methacrylate, 20 ml triethylene glycol monomethacrylate (Example 2) and 1 ml of Darocur 1173 (Ciba) was bubbled with argon for 10 min and poured into a mould for preparation of films of 1.6 mm thickness. The polymerization mixture was irradiated with Philips UV lamps of total output 120 W from a distance of 20 cm for 20 min. The obtained film was extracted with 30% ethanol and water.
- 10 From the swollen film were cut rings 21 mm in diameter.

Example 6

- A mixture of 30 ml of 2-hydroxyethyl methacrylate, 5 g 7-methacrylamidoheptanoic acid and 0.2 g Irgacure 2959 (Ciba) was bubbled with argon for 10 min and poured into a mould for
- 15 preparation of films of 1.6 mm thickness. The polymerization mixture was irradiated with Philips UV lamps of total output 120 W from a distance of 20 cm for 20 min. The obtained film was extracted with 30% methanol and distilled water.

Example 7

- 20 A mixture of 40 ml of 2-hydroxyethyl methacrylate, 10 ml 2,3-dihydroxypropyl methacrylate, 5 ml *N,N*-dimethacrylamide and 0.4 g Irgacure 2959 (Ciba) was bubbled with argon for 10 min and poured into a mould for preparation of films of 1.8 mm thickness. The polymerization mixture was irradiated with Philips UV lamps of total output 120 W from a distance of 20 cm for 25 min. The obtained film was extracted with 25% methanol and water
- 25 and dried.

Example 8

- A mixture of 30 ml of 3-hydroxypropyl methacrylate, 10 ml 2-methoxyethyl methacrylate, 15 ml of a mixture containing tetraethylene glycol monomethacrylate (Example 1) and 0.26 g
- 30 2,2'-azobis(2-methylpropanenitrile) was bubbled with argon for 10 min and poured into a mould for preparation of films of 1.6 mm thickness. The polymerization mixture was heated gradually to 48 – 62 °C for 11 h. The obtained film was extracted with 30% methanol and water and dried before use.

Example 9

A mixture of 40 ml of 2-hydroxyethyl methacrylate, 20 ml of diethylene glycol monomethacrylate and 0.38 g of 4,4'-azobis(4-cyanopentanoic acid) was bubbled with nitrogen for 11 min and poured into a mould for preparation of films of 1.6 mm thickness.

- 5 The polymerization mixture was heated gradually at 50 – 63 °C for 12 h. After taking out from the mould, the film was extracted with 30% methanol and water and dried.

Example 10

- A mixture of 30 ml of 1-vinylpyrrolidin-2-one, 20 ml 2-hydroxyethyl acrylate, 15 ml 4-hydroxybutyl methacrylate, 2 ml 2-[[[(vinylloxy)carbonyl]oxy]ethyl methacrylate, and 1 ml of Darocur 1173 (Ciba) was bubbled with carbon dioxide for 10 min and poured into a mould for preparation of films of 1.8 mm thickness. The polymerization mixture was irradiated with Philips UV lamps (120 W) from a distance of 22 cm for 15 min. The obtained film was extracted in a standard way.

15

Example 11

- A mixture of 25 ml of 2-hydroxypropyl methacrylate, 25 ml 2-hydroxyethyl methacrylate, 3 ml triethylene glycol dimethacrylate, 10 ml glycerol methacrylate (mixture of isomers), 10 ml of 2-acetoxyethyl methacrylate, and 1 g Irgacure 2959 (Ciba) was bubbled with nitrogen for 15 min and poured into a mould for preparation of films. The polymerization mixture was irradiated with Philips UV lamps (100 W) from a distance of 25 cm for 23 min. The obtained film was extracted in a standard way.

20

Example 12

- 25 A mixture of 50 ml of 2-hydroxypropyl methacrylate, 20 ml 2-hydroxyethyl methacrylate, 10 g *N*-(6-aminohexyl)methacrylamide and 0.7 g Darocur 1173 (Ciba) was bubbled with nitrogen for 15 min and poured into a mould for preparation of films (1.5 mm). The polymerization mixture was irradiated with a UV source (120 W) from a distance of 20 cm. The obtained film was washed in a standard way.

30

Example 13

A mixture of 20 ml of 2-hydroxyethyl methacrylate, 30 ml poly(ethylene glycol) monomethacrylate (Polyscience, Inc., $n = 200$), 2 ml diethylene glycol dimethacrylate and 0.7 ml Darocur 1173 (Ciba) was bubbled with nitrogen for 15 min and poured into a mould for

preparation of films (1.6 mm). The polymerization mixture was irradiated with a UV source (120 W) from a distance of 25 cm for 20 min. The monomer residues after polymerization were removed by extraction of the film with 30% ethanol and water.

5 Example 14

A mixture of 40 ml of 2-hydroxyethyl methacrylate, 10 ml 2-methoxyethyl methacrylate, 2 ml *N,N*-dimethacrylamide, 2.1 g *N*-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}acrylamide and 0.4 g Darocur 1173 (Ciba) was bubbled with argon for 10 min and poured into a mould for preparation of films of 1.6 mm thickness. The polymerization mixture was irradiated with
10 UV light as in Example 7.

Example 15

A mixture of 30 ml of 2-hydroxyethyl methacrylate, 15 ml 2-methoxyethyl methacrylate, 2 g *N*-{12-[(1,3-dioxoisindolin-2-yl)oxy]-12-oxododecyl}methacrylamide and 0.6 ml Darocur
15 1173 (Ciba) was bubbled with argon and poured into a mould for preparation of films of 1.6 mm thickness. The monomer mixture was irradiated with UV light as in Example 7.

Example 16

The polymer prepared according to Example 3 (rings 21 mm in diameter, cut in the swollen state, dry weight 5 g) was immersed in a solution of 3 g 4-nitrophenyl chlorocarbonate in 30
20 ml toluene, 5 ml pyridine and 10 ml acetone for 48 h. The polymer was washed with acetone three times (total 200,ml) and dried.

Example 17

5 g of the polymer prepared according to Example 9 was immersed in a solution of 3.5 g of 2-[(chlorocarbonyl)oxy]isoindoline-1,3-dione in 50 ml of an acetone-dioxane 1:1 mixture and 5 ml of triethylamine. After 24 h the solution was removed and the polymer washed
25 successively with dioxane and acetone and dried.

30 Example 18

10 g of the polymer prepared from 2-hydroxyethyl methacrylate (according to Example 4) was immersed in a solution of phosgene (10 g) in toluene (65 ml) for 24 h. After the reaction the polymer was washed three times with acetone and dried.

Example 19

1 g of the polymer according to Example 14 was mixed with a solution of 1.1 g of 2,2'-oxydiethan-1-amine dihydrochloride, 1.2 g sodium hydrogencarbonate, 5 ml water and 1.5 ml dimethyl sulfoxide was left reacting for 24 h. After finishing the reaction the polymer is washed with water, ethanol and water and dried.

Example 20

The polymer prepared according to Example 18 (6 g, rings 11 mm in diameter) was immersed in a solution of 5 g of hexamethylenediamine in 30 ml dioxane. After 24 h the solution was removed and the polymer was washed with a mixture of 30% methanol and 0.5% sodium hydrogencarbonate.

Example 21

A mixture of 30 ml of 2-hydroxyethyl methacrylate, 10 ml water, 0.3 g methylenebisacrylamide, 2 ml of a 10% solution of ammonium persulfate and 2 ml of 10% solution of *N,N,N',N'*-tetramethylethylenediamine in methanol was poured into a mold for preparation of films. The gel obtained after 24 h was washed with water and dried.

Example 22

1 g of the polymer prepared according to Example 14 as a 1.3 mm-thick film was immersed into a solution of 0.5 g of 4-(2-aminoethyl)aniline in 20 ml dioxane. The solution was removed after 48 h. the polymer was extracted for 2 days with a 5 % solution of sodium hydrogencarbonate in 30% methanol.

Example 23

The film prepared from a mixture of 10 g of 2-hydroxyethyl methacrylate and 0.7 g 6-methacrylamidohexanoic acid according to Example 6 (squares 2 x 2 cm, thickness 1.6 mm, weight 5 g) was swollen in a mixture of 5 ml of isopropyl alcohol and 15 ml water, the reaction mixture was cooled to 0 °C and 1.5 g of *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide was added. After 1 h, 3 ml of hexamethylenediamine was added. After 24 h at room temperature, the solution was removed and the polymer was extracted with 30% methanol containing 1 % of acetic acid and finally with a 1% solution of sodium hydrogencarbonate and water and dried.

Example 24

The rings (17 mm in diameter, 1.5 g) prepared according to Example 17 were covered with a solution of 0.35 g of *N*-(α -D-mannopyranosyl)glycinamide in 3 ml water and 1.5 ml dimethyl sulfoxide. The reaction was quenched after 2 days by washing with excess distilled water.

5

Example 25

A mixture of 1 g 4-nitrophenyl chlorocarbonate, 10 ml dioxane, 1 ml pyridine and 0.1 g 4-(dimethylamino)pyridine was added to the rings (diameter 18 mm, 5 g) prepared according to Example 13. After 24 h, the rings were washed three times with acetone, ethanol and water
10 and 0.5 g 6-aminoethyl α -D-mannopyranoside in a mixture of 5 ml water and 3 ml dimethyl sulfoxide was added. The reaction was quenched after two days by washing with 30% methanol and the polymer was extracted with a 5% solution of sodium hydrogencarbonate (for three days) and distilled water (for two days).

15 Example 26

Cultivation supports (thickness 1.3 mm, 2.1 g) prepared according to Example 10 were covered with a solution of 1 g 4-nitrophenyl chlorocarbonate, 10 ml dioxane and 2 ml pyridine. After 2 days, the polymer supports were washed with a mixture of acetone, dioxane and dichloromethane. The polymer was reacted with a solution of 0.4 g of 2-amino-2-deoxy- α -D-galactose in a mixture of 1 ml dimethylformamide and 3 ml water. The reaction was
20 quenched after two days by washing with water, a mixture of methanol – water (1:1) and water.

Example 27

25 The polymer supports 2x2 cm prepared according to Example 22 (thickness 1.8 mm, 5.6 g) were moistened with a solution of 2 g thiophosgene in 10 ml of acetone. The reaction was quenched after 2 days by washing the film three times with acetone and drying.

Example 28

30 To the polymer matrix prepared according to Example 18 (rings 16 mm in diameter, thickness 1.3 mm, 5 g) was added a solution of 1.2 g of methyl 6-amino-6-deoxy- α -D-mannopyranoside and 0.2 g 4-(dimethylamino)pyridine in 12 ml water and 3 ml dimethyl sulfoxide. The reaction was stopped after 2 days by washing the film with water and drying.

Example 29

A solution of 1 g *N*-[3-(dimethylamino)propyl]*N'*-ethyl-carbodiimide, 0.5 g 1-hydroxybenzotriazole and 0.5 g 5-carboxypentyl α -D-mannopyranoside in 20 ml water (first under cooling to 5 °C) was added after 30 min to a polymer film prepared according to Example 19 (5 g, rings 19 mm in diameter, thickness 1.8 mm). The reaction mixture was left standing at laboratory temperature for two days and the film was washed with acetone, methanol and water.

Example 30

The polymer film prepared according to Example 12 was overlaid with a solution of 1.3 g of 4-isothiocyanatophenyl α -D-mannopyranoside in 10 ml of acetone – isopropyl alcohol 1:1. After 2 days the polymer film was washed three times with acetone, twice with methanol, then with water and dried.

Example 31

The polymer prepared according to Example 20 (6 g, rings 11 mm in diameter) was covered with a solution of 1.8 g of β -galactopyranosyl-(1 \rightarrow 4)-D-glucono-1,5-lactone and 0.1 g 4-(dimethylamino)pyridine in 10 ml of dioxane – water 1:1. The reaction was stopped after two days and the film was washed with dioxane, methanol and water.

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Example 32

A polymer support prepared according to Example 17 (rings 15 mm in diameter, 5 g, thickness 1.8 mm) was added to a solution of 1.2 g of 2-aminoethyl α -D-mannopyranoside in a mixture of 5 ml dimethylformamide and 8 ml water. The reaction mixture was left standing for three days and then the support washed with water, 30% methanol and water.

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Example 33

Five rings of dry polymer film (thickness 1.1 mm, diameter 11 mm) prepared by radical polymerization according to Example 3 was immersed into a solution of 1 g 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl trichloroacetimidate dissolved under cooling (5 °C) in a mixture of 12 ml acetonitrile and 1 ml of boron trifluoride etherate as catalyst. The reaction mixture was cooled to 0 °C for 2 h under occasional shaking. The reaction was stopped by adding 20 ml of a 5% sodium hydrogencarbonate solution. The polymer was washed with water and dried.

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Example 34

The film according to Example 12 (3.7 g, ring diameter 18 mm, thickness 1.8 mm) was treated with a solution of 1.1 g of 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl isothiocyanate in 15 ml dichloromethane at laboratory temperature. After 24 h, the rings were washed with dichloromethane, methanol and water.

Example 35

Five rings of the film prepared according to Example 19 (ring diameter 18 mm, thickness 1.8 mm, 3.6 g) were overlaid with 15 ml of a dioxane solution of 1.2 g 4-isothiocyanatophenyl α -D-mannopyranoside. After 2 days, the rings were washed with dioxane, methanol and water.

Example 36

A polymer film according to Example 20 (ring diameter 19 mm, thickness 1.8 mm, 3.7 g) was immersed in a solution of 1.8 g 2-isocyanatoethyl α -D-mannopyranoside in a mixture of 10 ml acetone and 5 ml dioxane. After two days, the film was washed with dioxane, acetone and water.

Example 37

The film according to Example 10 (4.5 g, ring diameter 18 mm, thickness 1.2 mm) was immersed in a solution of 5-carboxypentyl α -D-mannopyranoside, 0.3 g *N*-[3-(dimethylamino)propyl]*N*-ethyl-carbodiimide and 0.2 g 1-hydroxy benzotriazole in a mixture of 15 ml dimethylformamide and 5 ml water. After 2 days, the solution was removed, the film was washed successively with dioxane, 5% acetic acid, 5% sodium hydrogencarbonate and water.

Example 38

A polymer film according to Example 36 (ring diameter 20 mm, thickness 0.9 mm, 3 g) was covered with a solution of 2 g 4-nitrophenyl chlorocarbonate in 10 ml dichloromethane. After two days, the film was washed with 3x50 ml dichloromethane and dried.

Example 39

A polymer film according to Example 38 (1 g, ring diameter 20 mm, thickness 0.9 mm) was immersed in a solution of 1 g of 3,3'-oxydipropan-1-amine in 10 ml of a mixture dioxane – isopropyl alcohol 1:1. After two days the product was washed three times with dioxane.

5 Example 40

The film rings prepared according to Examples 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35 and 36 were used for cultivation of keratinocytes [1-4]. In all cases the cultivation was more successful than on standard films prepared from 2-hydroxyethyl methacrylate. The films from Examples 24, 25 and 28 were better by 50-80 %, while the other exceeded pronouncedly
10 100 %.

Claims

1. Polymer carriers with bonded saccharides mannose, galactose or disaccharides where at the nonreducing end of the disaccharide is mannose or galactose, which are covalently bonded to polymer matrix through various types of spacers thereby forming polymer matrices suitable for cultivation of keratinocytes or temporary immobilization of biological systems with receptors for mannose and galactose of the general formula
- $$PM - [R - X - (R^1 - Y)_p - Z]_n,$$

where PM is a hydrophilic crosslinked polymer prepared by radical polymerization of a mixture containing

- (a) 1-99 wt.% of a monomer or a monomer mixture,
- (b) 0.1-10 wt.% of a crosslinker or crosslinker mixture,
- (c) 0.01-10 wt.% of a radical initiator,
- with or without (d) 0.1-40 wt.% of a solvent or solvent mixture

and the generally formulated structure

$-[R - X - (R^1 - Y)_p - Z]_n$ formed by additional modification of polymer matrix,

where R is independently selected from the group: covalent bond, $-(CH_2)_a-$, $-OCH_2CH_2-$, $-(OCH_2CH_2)_b-$, $-C_6H_4-O-$, $-C_6H_4-CO-$, $-NH-C_6H_4-CO-$, $-C_6H_4-NH-$, $-O-C_6H_4-O-CH_2CH_2O-$,

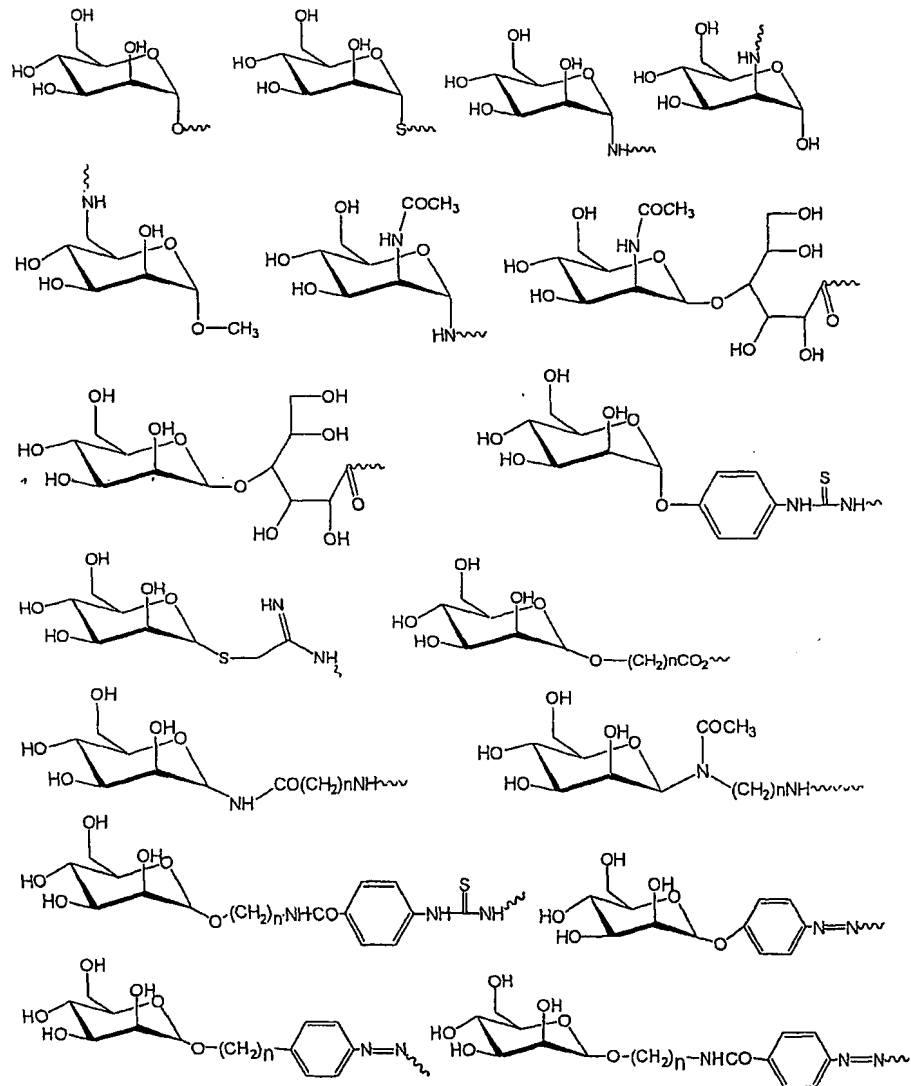
X is independently selected from the groups: covalent bond, $-O-$, $-NR^2-$, $-CO-O-$, $-CO-NH-$, $-NH-CO-$, $-O-CO-O-$, $-NH-CS-NH-$, $-NH-CO-NH-$, $-NH-CH_2CH_2CONH-$, where R^2 is $-H$, alkyl $C_1 - C_4$ or acetyl,

R^1 is independently selected from the groups: covalent bond, $-(CH_2)_a-$, $-OCH_2CH_2-$, $-C_6H_4-O-$, $-(OCH_2CH_2)_b-O-$, $-C_6H_4-CO-$, $-NH-C_6H_4-CO-$,

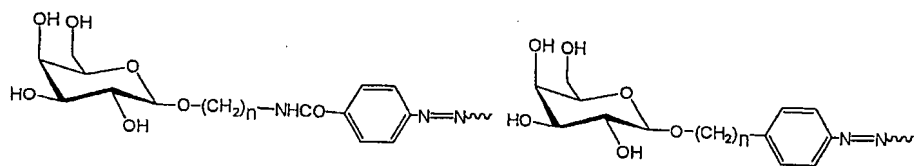
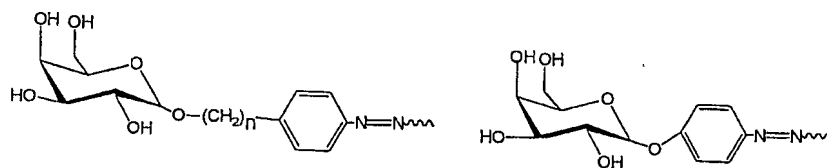
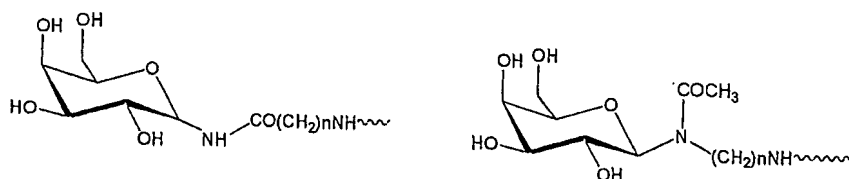
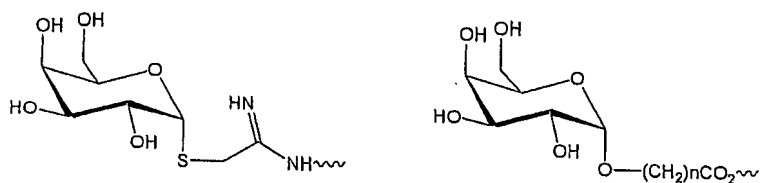
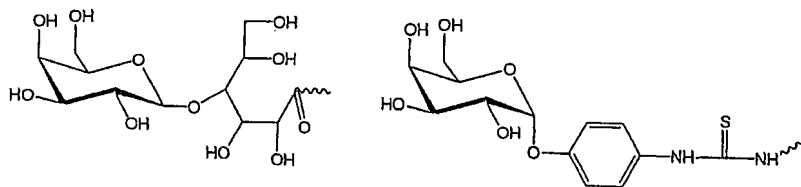
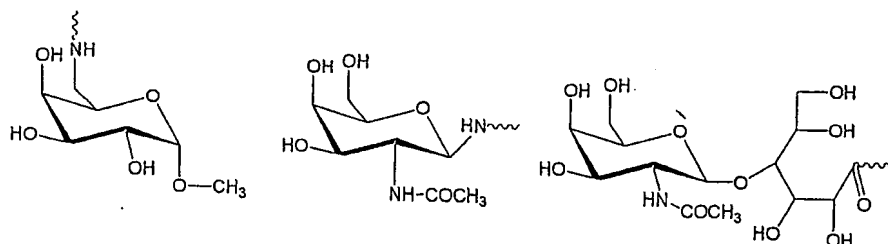
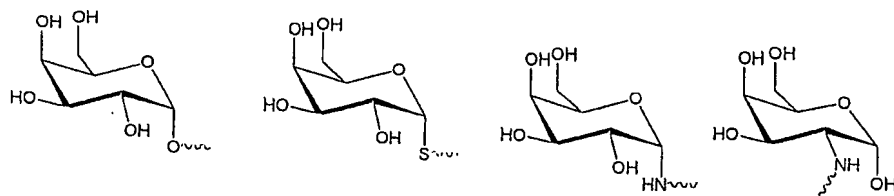
Y is independently selected from the groups: covalent bond, $-N(COCH_3)-$, $-NH-CS-NH-C_6H_4-O-$, $-NH-CS-NH-(CH_2)_a-C_6H_4-O-$, $-NH-CS-NH-(CH_2)_a-O-C_6H_4-O-$, $-NH-C_6H_4-O-$, $-NH-C_6H_4-CO-$

where subscript a ranges from 1 to 12, b from 1 to 200, p from 0 to 20, subscript n is selected so that the saccharide concentration ranging from 1×10^{-4} to 0.3 g per gram of polymer matrix is provided,

Z is independently selected from a group of mannose, galactose and lactose derivatives, which are given as structural formulae, where the wavy line indicates the attachment of saccharide wherein the mannose derivatives are

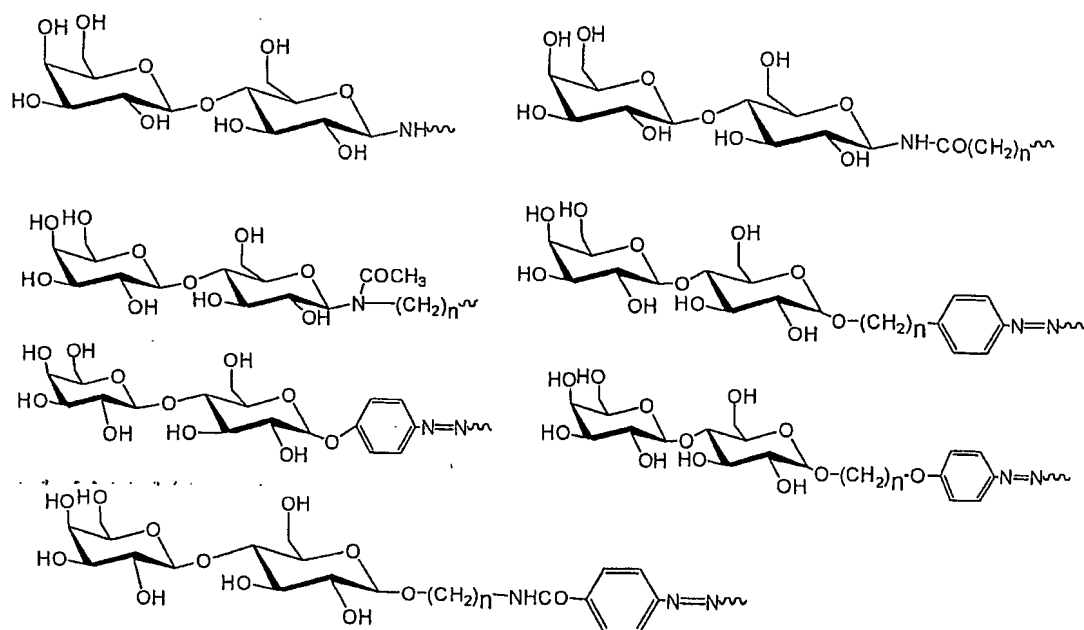


galactose derivatives are



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and lactose derivatives are



2. Polymer carriers with bonded saccharides mannose, galactose or disaccharides, where
- 5 at the nonreducing end of the disaccharide is mannose or galactose, which are covalently bonded to polymer matrix through various types of spacers thereby forming polymer matrices suitable for cultivation of keratinocytes or temporary immobilization of biological systems with receptors for mannose and galactose according to Claim 1, where the polymerization mixture for hydrophilic polymer PM contains, individual or
- 10 in a mixture, monomers selected from the group 2-hydroxyethyl acrylate, 2-hydroxyethyl methacrylate (HEMA), 2-(2-hydroxyethoxy)ethyl acrylate, 2-(2-hydroxyethoxy)ethyl methacrylate (DEGMA), tri-, tetra- and poly(ethylene glycol) monoacrylate and methacrylate, glycerol acrylate and methacrylate, 2-hydroxypropyl acrylate and methacrylate, ω -hydroxyalkyl acrylates and methacrylates, (ω -
- 15 hydroxyalkyl)acrylamides and -methacrylamides, (ω -aminoalkyl)acrylamides and -methacrylamides, glycidyl acrylate and methacrylate, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]acrylamide and -methacrylamide, acrylic and methacrylic acids, ω -acrylamido- and ω -methacrylamidoalkanoic acids, 4-vinylbenzoic acid, acrylamido- or methacrylamidobenzoic acids, *N*-alkylacrylamides and -
- 20 methacrylamides, 2-(4-vinylphenoxy)ethan-1-ol, vinyl acetate, 2-(methylsulfanyl)ethyl acrylate and methacrylate, 2-(methylsulfinyl)ethyl acrylate and methacrylate, 2-(methylsulfonyl)ethyl acrylate and methacrylate, 2-methoxyethyl

acrylate and methacrylate, 2-acetoxyethyl acrylate and methacrylate, methyl acrylate and methacrylate.

3. Polymer carriers with bonded saccharides mannose and galactose or disaccharides, where at the nonreducing end of the disaccharide is mannose or galactose, which are covalently bonded to polymer matrix through various types of spacers thereby forming polymer matrices suitable for cultivation of keratinocytes or temporary immobilization of biological systems with receptors for mannose and galactose according to Claims 1 and 2, where the polymerization mixture for hydrophilic polymer PM contains a crosslinker or a crosslinker mixture selected from a group of compounds ethylene diacrylate and dimethacrylate, diethylene glycol and oligo(ethylene glycol) diacrylates and dimethacrylates, *N,N'*-ethylenediacrylamide and *N,N'*-ethylenedimethacrylamide, 1,3-divinylurea, 1,1'-divinyl-3,3'-(ethane-1,1-diyl)di(pyrrolidin-2-one).
4. Polymer carriers with bonded saccharides mannose and galactose or disaccharides, where at the nonreducing end of the disaccharide is mannose or galactose, which are covalently bonded to polymer matrix through various types of spacers thereby forming polymer matrices suitable for cultivation of keratinocytes or temporary immobilization of biological systems with receptors for mannose and galactose according to Claims 1 - 3, where the polymerization mixture for hydrophilic polymer PM contains a radical initiator generating radicals by heating, such as an azo initiators, diacyl peroxide or other type of peroxo compound, an initiator generating radicals by UV radiation or a redox initiator, which generates radicals through an oxidation-reduction reaction.
5. Polymer carriers with bonded saccharides mannose, galactose or disaccharides, where at the nonreducing end of the disaccharide is mannose or galactose, which are covalently bonded to polymer matrix through various types of spacers thereby forming polymer matrices suitable for cultivation of keratinocytes or temporary immobilization of biological systems with receptors for mannose and galactose according to Claims 1 - 4, where, as a constituent of the polymerization mixture for hydrophilic polymer PM, a solvent or solvent mixture is used selected from compounds such as water, alcohols such as methanol, ethanol, ethylene glycol, glycerol, dimethylformamide, dimethyl sulfoxide, poly(ethylene glycol)s, esters of aliphatic acids, monomethyl or dimethyl

ethers of ethylene glycol.

6. Polymer carriers with bonded saccharides mannose, galactose or disaccharides, where at the nonreducing end of the disaccharide is mannose or galactose, which are covalently bonded to polymer matrix through various types of spacers thereby forming polymer matrices suitable for cultivation of keratinocytes or temporary immobilization of biological systems with receptors for mannose and galactose according to Claims 1 - 5, prepared by a properly chosen modification reaction or a series of consecutive reactions, where the modification reagent is selected from the group of compounds activating on the polymer carrier
- a) hydroxy or amino group, where as an activator can be used dichlorides or ester chlorides of dicarboxylic acids of general formula HOOC-Q-COOH , where Q is a bifunctional aliphatic chain, branched aliphatic chain, cycloalkanediyl, cycloalkenediyl, benzenediyl, furandiyl, oxydiethylene, diisocyanates of the general formula OCN-T-NCO , where T is a divalent aliphatic chain, cyclohexane-1,4-diyl, methylenedi(1,4-phenylene), oxydi(1,4-phenylene), methylenedi(cyclohexane-1,4-diyl), further bromocyanogen, phosgene, diphosgene, thiophosgene, chlorocarbonates of aliphatic alcohols, branched aliphatic alcohols, cyclic alcohols, further *N,N*-carbonyldiimidazole or other derivatives of carbonic acid commonly used in the field;
- b) carboxyl group, where as an activator can be used thionyl chloride, mixed anhydrides, active esters, carbodiimides under catalysis with *N*-hydroxysuccinimide, 1-hydroxybenzotriazole, acid hydrazides, acid azides.
7. Polymer carriers with bonded saccharides mannose, galactose or disaccharides, where at the nonreducing end of the disaccharide is mannose or galactose, which are covalently bonded to polymer matrix through various types of spacers thereby forming polymer matrices suitable for cultivation of keratinocytes or temporary immobilization of biological systems with receptors for mannose and galactose according to Claims 1 - 6, prepared by final modification of the matrix with mannose, galactose or lactose derivatives such as amino derivatives, isothiocyanates, trichloroacetimidates, aldehydes, reactive carboxyl derivatives, activated carboxylic esters bonded to saccharides.

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
12 August 2004 (12.08.2004)

PCT

(10) International Publication Number
WO 2004/067732 A3

(51) International Patent Classification⁷: C12N 5/00, C08F 220/00, 8/32, C12N 5/00, C08F 220/00

(21) International Application Number:
PCT/CZ2004/000005

(22) International Filing Date: 26 January 2004 (26.01.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PV 2003-251 27 January 2003 (27.01.2003) CZ

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): LABSK, Jirí [CZ/CZ]; Na Vypichu 1956/28, 160 00 Praha 6 (CZ).

(74) Agent: SEMBEROVÁ, Dana; Patent and License Services ASCR, Politick ch veznu 7, 111 21 Praha 1 (CZ).

(88) Date of publication of the international search report:
18 November 2004

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYMER CARRIERS WITH BONDED SACCHARIDES FOR IMMOBILIZATION OF BIOLOGICAL SYSTEMS

(57) Abstract: The solution concerns polymer carriers with bonded saccharides for immobilization of biological systems, where at the nonreducing end of a disaccharide is mannose or galactose that are covalently bonded to polymer matrix through various types of spacers, which enables a better contact of a saccharide molecule with receptors of biological systems.

WO 2004/067732 A3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CZ2004/000005

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/00 C08F220/00 C08F8/32 C12N5/00 C08F220/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C08F C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

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Date of the actual completion of the international search

27 July 2004

Date of mailing of the international search report

01/10/2004

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

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PCT/CZ2004/000005

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CZ2004/000005

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